Median Raphe Nucleus of adult rat: sexual dimorphism and effects of female gonadal steroid deprivation

Seyed Behnamedin Jameie[1] , Fatemeh Shahnam[2], Hossein Kelarestaghy[2], Shayesteh Mehdinejadiani[2], Mahdiyeh Kerdari [1], Azita Kouchmeshky [1], Mona Farhadi [3]

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Abstract

Introduction: During the last decades it is shown that the gonadal steroids affects the nervous system. Gonadal steroids easily cross the brain blood barrier and insert their effects on morphometric parameters of the certain areas of the mammalian brain despite their direct or indirect roles in sexual behavior, the effects that generally called sexual dimorphism (SD). Among the different brain neurotransmitter system, serotonergic system is of more inertest to study for SD due to its extensive projections and the role of its neurotransmitter, serotonin (5HT), in different physiological and pathological conditions including neuronal firing, human emotional control, and affective disorders. Although the fact that 5HT is affect by the sexual gonadal steroids, it is not known whether the nuclei related to this system also influenced by gonadal hormones. Median raphe nucleus (MRN) is the largest one among the other nuclei in human brain and involves in many important functional roles. There are not enough evidences regarding the SD in median raphe nucleus of mammalian brain and also the influence of gonadal steroids on its morphometric parameters. The present study was supposed to answer the mentioned doubtfully questions.

Material & Methods: Sixty adult male and female Sprague-Dawelly rats (200-230g) were used in this study. The animals randomly were divided to four groups including normal female group, normal male group, ovariectomized group (OVX) and sham surgery group. For SD the animals of normal male and female groups were compared and for the study of the effects of female gonadal steroids the normal female group compared with OVX group. The animals perfused and fixed transcardially, brain stem was removed, coronal sections were obtained and processed for light microscopic study. Nissl and Golgi staining used for study morphometric and neuronal morphologic parameters of MRN. Data analyzed and the results presented by means± SD.

Results: Based on our findings MRN showed sexual dimorphism and gonadal steroid deprivation via ovariectomy significantly influenced certain morphometric parameters of MRN.

Conclusion: According to the results of this study, sexual dimorphism of MRN and the influence of female gonadal steroids on serotonergic nuclei should consider in normal and pathological conditions.

Key words: Median raphe nucleus, female gonadal steroids, ovariectomy, sexual dimorphism

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[1] *PhD Basic Science Lab, Basic Science Department, Faculty of Allied Medicine, Department of Anatomy, Faculty of Medicine and Cellular & Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran.* behjam@yahoo.com & behnamjameie@iums.ac.ir

[2] *Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

[3] *PhD Department of Biology, Faculty of Agriculture, Islamic Azad University Saveh Branch, Saveh, Iran*

Introduction

In living organisms ranging from fungi to humans many structural and functional biological properties are determined by sexual and non-sexual steroid hormones.[1] As a manner of fact when the behavior of the sexes differs markedly, there must be sex differences in brain structure mediating those behavioral differences. Historically it was believed that there is a unidirectional relation between brain and reproductive system, accordingly only certain areas of the nervous system that directly involves in control of reproductive behavior are different between male and female. During the last decades it has been shown that the sex steroid hormones are small molecules that easily cross blood brain barrier and influence certain parts of the mammalian nervous system at different anatomical and functional levels.[1] The effects of gonadal steroids on the nervous system leads to differences between male and female brain and spinal cord areas, which known as SD or sexual dimorphism in nervous system.[2] It is now known that levels of circulating sex steroid hormones during development and in adult hood play a critical role in determining physiology and behavior.[3,4] The neural mechanisms underlying hormonal effects on brain function and morphology have been the subject of numerous studies. Traditionally, steroid hormone effects have been divided into 2 categories including organizational or genomic and activational or non-genomic effects.[5] Whereas organizational effects have been defined as permanent structural changes induced by hormones during development, activational effects have been defined as the turning on or off of previously established neuronal circuits in adulthood.[5,6] For the regions of the CNS which directly involve in reproductive behavior SD varies from differences in the size of individual neurons, dendritic branching patterns, and synaptic organization, to dramatic dimorphisms in the length and volumes of the nuclei. Numerous findings show that these morphometric parameters are not limited to these areas and have been reported in regions not necessarily involved in reproductive behavior.[7,8] Since the morphologic characteristics of neurons have been shown to influence the functional properties of the neurons, it is likely that these hormone induced structural changes contribute significantly to the activation of neural circuits necessary for certain behaviors.[9] Regarding to the critical periods for sex steroidal effects on structure and function of NS, recent findings emphasize that these effects are not limited to the prenatal and before puberty period but also the level of circulatory sexual hormones in all developmental stages even in adult animal has an important role in

physiology and behavior by affecting the cellular, molecular and microscopic parameters of the neurons nuclei and neural circuits activity.[10, 11] Some of these findings mostly obtained from sex steroidal deprivation studies in adult animals that demonstrated any manipulation of sex steroid hormone levels in adulthood can induce reversible or non-reversible dramatic macroscopic and microscopic changes in certain regions of the central nervous system,[12,13,14] such as neurons of adult avian song system,[15,16] corpus callosum and anterior commisure,[17,18,19], bulbocavernous spinal nucleus,[19,20] spinal motoneurons,[21] rat purkinje cell,[22] SDN-POA of hypothalamus,[23,24,25] hippocampal pyramidal cells,[26] bed nucleus of human stria terminalis,[27] nigrostriatal dopamine neurons,[28] rat arcuate nucleus,[29,30,31] human median raphe nucleus,[32] substantia nigra[33] and dendritic spine density of the rat dorsal raphe nucleus/DRN.[34] The serotonergic system is the most expansive and complex anatomic and neurochemical system in mammalian brain, its projections receive to many areas of the brain, the nuclei and its neurotransmitter are involved in numbers of important physiological and pathological functions.[35,36] It is reported that the serotonergic system is particularly sensitive to female gonadal steroids as any alterations in level of female sex hormones dramatically changes serotonergic system physiology.[37,38] Among the serotonergic nuclei, DRN and MRN received the most attention, SD has been reported for DRN but not for MRN, it is also not known whether the female sex steroid deprivation could influence on MRN or not. In the present study MRN as the largest raphe nucleus in human is evaluated firstly for SD and in second for the effects of female gonadal steroid deprivation.

Materials and Methods

Sixty female and male adult Sprague_Dawley rats (200-250 g) were used in this study. The animals prepared from Razi Institute (Karj, Hesark Institute,) and were maintained in standardized environment with respect to both photoperiod cycle (10 h light, light at 06.00) and temperature (22º C). Food and water were provided ad libitum throughout the experiments. All the procedures were approved by local committee of Ethic and Research of IUMS. The animals randomly divided to four groups (n=15) including intact female, intact male, ovariectomized rats (OVX), sham surgery. In order to study SD in MRN the normal male and female groups were compared and for the study of the effects of female gonadal steroids deprivation the normal female group compared with OVX group. The morphometric parameters including length, volume, total neuronal number, density and morphology of the neurons of MRN were considered in this study.

For ovariectomy female rats were anesthetized with IP injection of mixture of Ketamine (100mg/kg)and Xylasine(1/8) , bilateral ovariectomy was done by midline ventral incision, the ovaries removed, remaining uterus ligated . For sham surgery group the animals under went same surgical procedure without removing the ovaries. One month of interval time considered for both groups. For Nissl staining the animals were perfused and fixed transcardially with aldehyde solutions (1.25% Glutaraldehyde, 4% Paraformaldehyde in Phosphate Buffer 0.1M pH 7.4) followed by 10% sucrose solution in Phosphate Buffer for cryo-protection. The brain stem was removed and maintained in graded 10%, 20% and 30% sucrose solution in Phosphate Buffer 0.1M pH 7.4 in 4°c for 3 days. By using freezing microtome, coronal sections of 30µm sections were obtained and processed for Nissl staining. Golgi-impregnation method was done for study the morphology of the neurons of MRN as follows, all the rats were deeply anesthetized with intraperitoneal injection of same anesthetic mixture and transcardially perfused with 300 ml 1% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate-buffered saline (PBS; pH 7.6) followed by 450 ml of second fixative containing 6% potassium dichromate, 6% chloral hydrate and 10% formaldehyde in 0.1M phosphate-buffered saline (PBS; pH 7.6). Brain stems were removed, blocks of 3 mm thick containing DRN prepared and postfixed in the second fixative for 24 h. Then, the blocks put in the solution of 3.5% potassium dichromate in distilled water for 48h, after that stored in 1.5% silver nitrate for 72 h in dark room. Dehydration and paraffin embedding were done and by using a rotatory microtom coronal sections of 80µm thickness were cut, mounted and coversliped. Light microscopic study was done and by using Olysia Bioreport software morphometric parameters of MRN including length, volume, total neuronal count and neuronal density was studied. The morphology of the neurons of MRN also studied by light microscopic. All the slides containing Golgi- impregnated and Nissl staining brain stems were coded prior to quantitative analysis. The code was not broken until after the analysis was completed. By using SPSS 15, the means of variable were determined and these data were subjected to 1-way ANOVA analysis, the results were presented in mean± SD. The $Pv<0.05$ was considered as significant.

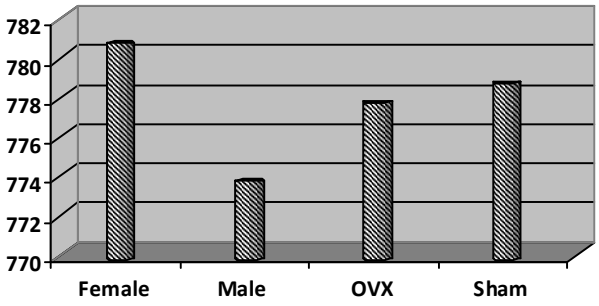
Results

In the present study the location of MRN in coronal sections of brain stems was defined based on Paxinos and Watson atlas of Rat Brain, according to the atlas the MRN is located ventral to decussation of superior cerebellar peduncles (xscp) and medial longitudinal fasiculus, the nucleus extended

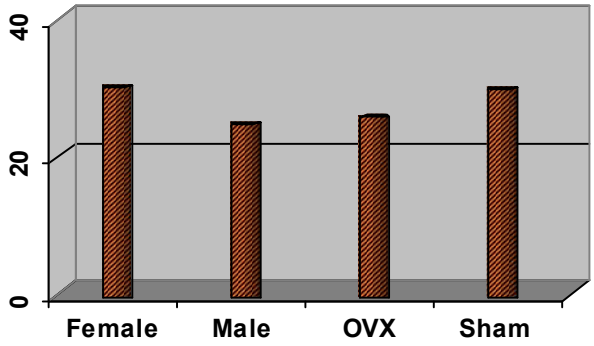
rostrocaudaly from xscp appears to pontine reticular nucleus (PnC) appears in sections. MRN also is surrounded by a very well defined vascular ring that helps us to define the border of the nucleus; the mentioned ring was recognizable in whole length of MRN that separates MRN from paramedian raphe area (Fig 1 A & B). Two types of neural staining including Nissl and Golgi were used in this study; the results respectively are as follows.

I- Results of Nissl staining

Nissl staining was used to study and compare the morphometric parameters of MRN including rostrocaudal length, total volume, number and density of the neurons among male, female, OVX and sham groups. For SD we compared the mentioned parameters between male and female rats. Our findings showed SD in length, volume and number of the neurons of MRN Total volume of MRN (fig.1 and tables 1-3). According to our data the length, volume and number of the neurons of MRN of female rats was larger and higher than male rats (graph 1-3).



Graph1: MRN volume



Graph 3: MRN neuronal number

For the volume and number of the neurons of MRN the sex differences was considered significant with Pv less than 0.05 (Pv = 0.0001, Pv =0.02 respectively). Regarding the rostrocaudal length of the MRN, our data showed non-significant longer length in MRN of female rats than male (Pv=0.65). To study of the effects of female gonadal steroid deprivation the results obtained from female rats considered as control group and compared with OVX and sham groups. Our findings showed significant decrease of the numbers of the neurons of MRN in OVX rats comparing to normal and sham animals (fig. 3 A-F). For the volume of MRN there was also significant decrease of volume of MRN between OVX rats with sham and normal female (Pv < 0.05). Length of MRN of OVX rats non-significantly is less than normal female and sham animals (table 1-3, graph 1-3).

Group	Mean	SEM
Female	176.2	3.955
Male	150	3.317
OVX	164.8	2.154
Sham surgery	180.8	2.557

Table1: MRN volume (10^{-3} mm^3)

Group	Mean	SEM
Female	30.86	0.9011
Male	25.46	0.5454
OVX	26.50	1.0302
Sham surgery	30.52	0.8438

Table3: Total cell number

II- Result of Golgi staining

We used Golgi staining only to study and compare the morphology of the neurons of MRN among the male, female and OVX groups. As MRN is surrounded by a recognizable vascular ring so light microscopic examination of Golgi-impregnated tissue revealed reliable and consistent neuronal staining throughout the MRN of all brain stems. In particular, three neuronal populations of MRN including polygonal, fusiform and round neurons were always well represented and easily identifiable in all parts of MRN of the Golgi stained sections of all groups (Fig. 3 A-F). Same population from morphological point of view was seen in male, female and OVX animals. We did not compare the number, density and fine structure of the neurons among the groups.

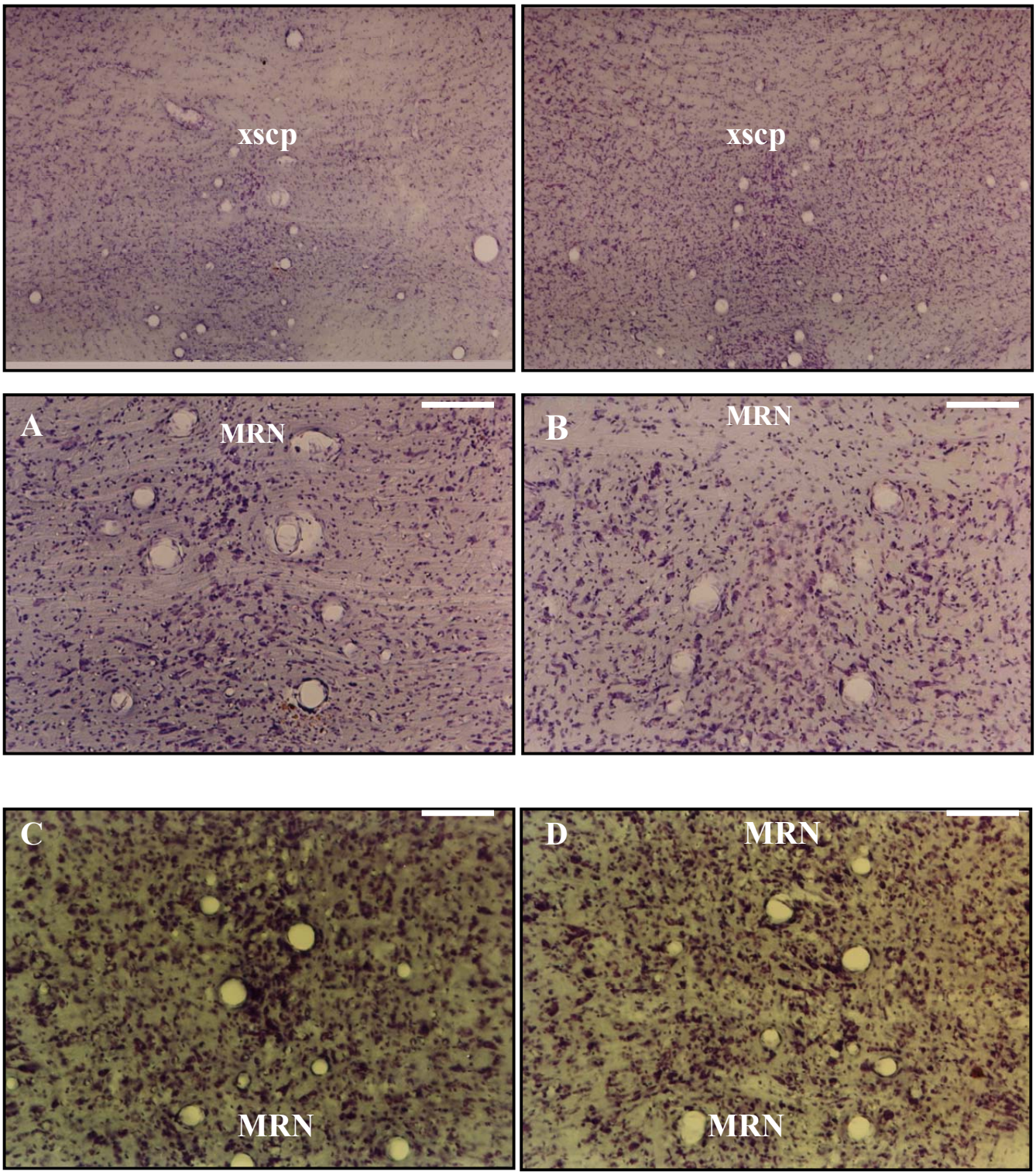


Fig 3: Photomicrographs of MRN. Intact female (A-C-E) , OVX (B-D-E) Scale bar for A & B 50 µm, C & D 30 µm, E & F 10 µm

Discussion

Results of the present study exactly showed SD in MRN, it is also showed that short-term female sex steroid deprivation through ovariectomy resulted in significant changes of morphometric parameters of MRN compared with that of intact females. SD has been reported for certain various nuclei of the brain in rodent, mammalinas, primates and humans based on morphometeric parameters including volume, length, number and density of the neurons,[13,28,39] In most of the cases the SD parameters in male nuclei such as DRN,[34] SDN-POA,[25] bed nucleus of human stria terminalis,[27] and rat arcuate nucleus,[31], are more dominanet than female. To our knowledge the only exception among these sexually dimorphic nuclei with dominacy in female than male reported in 1996 by Gorski et al is AVPN-SDN.[24]. There are few reports regarding SD in MRN, Cordero et al in 2000 reported SD in number and proportion of neurons in the human MRN via postmortem study, they showed male dominancy in MRN. No reports exist for SD in rodents, our findings not only showed SD in rat MRN but also female dominancy, which is different from our previous study for rat DRN that showed SD with male dominancy.[34] To explain our findings for SD of MRN attention to cellular and molecular mechanisms of sex steroidal effects on nervous system is needed. Numerous researches over the last decades has provided evidences for extensive steroid-dependent plasticity in the adult brain. Neuroactive steroid compounds apply to those steroids that have biological effects or activities in nervous system which may originate from various sources: a) the endocrine system mainly adrenal and sexual glands,[40, 41] b) the nervous system itself where steroids can be synthesized de novo,[28,39,42], c) exogenous from various environmental sources.[2,43] Sex steroids regulate neuronal function by influencing neurons via steroid receptors which have been defined in many parts of the brain, either involving in reproductive behavior or not.[28,44] Neurons responsive to gonadal steroids at least may have three types of steroids receptors acting throughout different mechanisms: 1) the well known intracellular steroid receptors responsible for steroidal genomic action,[45,46,47] that once activated act as transcription factors and may trigger gene expression and are typically responsible for late and long lasting neuronal response[2,9.48,49] 2) effects mediated by membrane receptors for non-genomic actions, 3) the recently described membrane steroidal receptors[50,51] which may be coupled directly to membrane ion channels or second messengers system that elicit rapid and transitory changes on neural structure.[52,53,54] In the case of steroidal effects on the serotonergic nuclei, it seem to be linked to the presence of esterogen and progestin-sensitive

neurons in the midbrain raphe nuclei and/or as well as possibly nongenomic actions in brain area to which serotonin neurons project their axons.[6] In our beliefs these considerations are not enough to explain different findings for SD of DRN and MRN in rats. There might be other mechanisms are still unknown and needs more reseach. For the results of the effects of sex steroid deprivation, there are numerous evidences that show female sex steroid deprivation leads to dramatical changes of morphometric parameters of certain nuclei in rodents and primates brain. Gorski et al (1996) demonstrated that OVX in rats significantly decreases the length and volume of sexual dimorphic nucleus of AVPN-POA.[24] Sexual dimorphic of dopaminergic neurons of substantia nigra have been frequently studied. Leranth et al in 2000 reported that OVX and gonadal steroids deprivation in female monkeys after 30 days will cause a 30% decrease in these cells and steroid treatment will not compensate it, but it is compensating with steroid prescription after 10 days.[28]. Jameie et al in 2004 reported same results for DRN of OVX rats, that followed OVX the number, density of denderitic spines and length of denderits significantly reduced.[34] Regarding these data neuroprotective properties suggested for female sex steriods. Growing evideces show that there are potent mechanisms to explain neuroprotective function of estrogens and preventing of apoptosis is one of these mechanisms[2,11,28] Low level of circulatory female sex steroidal hormones following OVX might induces apoptosis that in turn leads to morphometeric changes of the nuclei. The exact mechanisms of this phenomenon is still unknow and needs more reseach.

Conclusion

In summary, the cross talk between sex steroidal hormones and central nervous system play important roles in regulating behaviors either physiologic or pathologic. This interaction should consider in diagnosis, clinical and theraputical plan.

References

1- Kawata M. Roles of steroid hormones and their receptors in structural organization in the nervous system. Neurosci Res 1995; 24: 1-46.
2- Melcangi RC,& Panzica G . Neuroactive steroids: An update of their roles in central and peripheral nervous system. Psychoendocrinology2009; (11): 202-209.
3- Breedlove M. Sexual dimorphism in the vertebrate nervous system. J Neurosci1992; 12(11): 4133-4142.
4- Kuhl H. Influence of the ovarian cycle on the central nervous system. The Umsch 2002; 59(4): 175-81.
5- McEwen BS. Multiple ovarian hormone effects on brain structure and function. J Gend Specif Med 1998; 1(1): 33-41
6- McEwen BS. Estrogen actions throughout the brain. Recent Prog Horm Res 2002;57:357-384.
7- Morris JA., Jordan CL., & Breedlove M . Sexual differentiation of the vertebrate nervous system. Nature Neurosci 2004; 7(10): 1034-1039.
8- Gorski RA. Sexual dimorphism in the brain. Journal of the American academy of child and adolescent psychiatry1998; 72:33-35.
9- Arnold AP., & Gorski RA. Gonadal steroids induction of structural sex differences in the CNS. Annu. Rev. Neurosci 1984; 7: 413-442.
10- Bodo, C. A role for the androgen receptor in the sexual differentiation of the olfactory system in mice. Brain Res. Rev 2008 57:321-331.
11- Jordan CL, & Doncarlos L. Androgens in health and disease: An overview. Horm Behav2008; 53: 589-59.
12- Prince KN., Prince JS., Kinghorn EW., Donovan EF., & Rhees RW. Effects of sexual behavioral manipulation on brain plasticity in adult rats. Brain Res Bulletin 1998; 47(4): 349-355.
13- Keefe DL: Sex hormones and neural mechanisms. Arch Sex Behav 2002; 31(5): 401-3.
14- Genazzani AR., Monteleone P., & Gambacciani M. Hormonal influence on the central nervous system. Maturitas 2002; 30(43 Suppl 1): S11-7.
15- DeVoogd T., & Nottebohm F. Gonadal hormones induce dendritic growth in the adult avain brain. Science 1981; 214: 202-204.
16- DeVoogd T., Nixdorf B., & Nottebohm F. Synaptogenesis and changes in synaptic morphology related to acquisition of a new behavior. Brain Res 1985; 329: 304-305.
17- Allen LS., & Gorski RA. Sexual dimorphism of the anterior commissure and massa intermedia of the human brain. J. Comp. Neurol1991; 312: 97-104.
18- Allen LS., Richey MF., Chai YM., & Gorski RA. Sex differences in the corpus callosum of the living human being. J. Neurosci 1991; 11(4): 933-942.
19- Matsumoto A. Androgen stimulates neuronal plasticity in the perineal motoneurons of aged male rats. J. Com. Neurol 2001; 430: 389-395.
20- Kurz EM., Sengelaub DR., & Arnold P. Androgens regulate dendritic length of sexually dimorphic mammalian motor neurons in adulthood.
Science 1986; 232: 395-396.
21- Hebbeler SL., Verhovshek T., & Sengelaub. Ovariectomy attenuates dendritic growth in the hormone-sensitive spinal motoneurons. J Neurobiol 2001; 48(4): 301-314.

22- Sakamoto H., Ukena K., & Tsutsui K. Dendritic spine formation in response to progesterone synthesized de novo in the developing purkinje cell in rat. Neurosci Lett 2002; 322(2): 111-5.
23- Bloch GL., & Gorski RA. Estrogen/ progesterone treatment in adulthood affects the size of several components of the medial preoptic area in the male rat. J.Com. Neurol 1988; 275: 613-622.
24- Gorski RA, Davis EC, & Shryne JE. Structural sexual dimorphism in the AVPN nucleus of the rat hypothalamus are sensitive to gonadal steroids perinatally, but develop peripubertally. Neuroendocrinology. 1996; 63: 142-148
25- Gorski RA. Cytoarchitectonic analysis of the SDN-POA of the intact and ovariectomized rat. J Comp Neurol. 1998; 275(4):604-612
26- Gould E., Woolley CS., Frankfurt m.& McEween Bs. Gonadal steroids regulates dendritic spine density in hippocampal pyramidal cells in adulthood. J. Neurosci 1990; 10(4): 1286-1291.
27- Chung WC., De Vries Gj., & Swaab DF. Sexual differentiation of the bed nucleus of the stria terminalis in humans may extend into adulthood. J. Neurosci 2002; 22(3):1027-33.
28- Leranth C., Roth RH., Elsworth JD., Naftolin F., & Redmond DE. Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implication for parkinson's disease and memory. J. Neurosci 2000; 20(23): 8604-8609.
29- Keefe D., Garcia-Segura LM., & Naftolin F. New insights into estrogen action on the brain. Neurobiology of Aging 1994; 15(4): 495-497.
30- Mong JA., Glaser E., & McCarthy MM. Gonadal steroids promote glial differentiation and after neuronal morphology in the developing hypothalamus in a regionaly specific manner. J.Neurosci 1999; 19(4): 1464-1472.
31- Mong J., Roberts RC., Kelly JJ., & McCarthy M. Gonadal steroids reduce the density of axonospinous synapses in the developing rat arcuate nucleus: An electron microscopy analysis. J. Com. Neurol2001; 432:259-267.
32- Cordero ME., Valenzuela Cy., Torres R., & Rodriguez A. Sexual dimorphism in number and proportion of neurons in the human median raphe nucleus. Developmental Brain Research 2000; 124:43-52.
33- Veliskova J., & Moshe SL. Sexual dimorphism and developmental regulation of substantia nigra function. Ann. Neurol 2001; 50(5): 596-601.
34- Jameie SB., Noyan Asharaf MH., & Behzadi G. Ovariectomy reduces the denderitic spine density of the dorsal raphe neurons in the aduly rat. Arch Iranian Med. 2004; 7(2): 122-127
35- Jacobs BC., & Azmitia EC.. Structure and function of the brain serotonin system. Physiological Reviews 1992; 72(1): 165-229.
36- Mcentee WJ., & Crook TM. Serotonin, memory and the aging brain. Psychopharmacology 1991; 103: 143-149.
37- Munaro NL. The effects of ovarian steroids on 5HT neuronal activity. Neuroendocrinology 1978; 26: 270-276.
38- Matsuda Y., Hirano H., & Watanabe Y. Effects of estrogen on acetylcholine release in frontal cortex of female rats: involvement of serotonergic neuronal ystem. Brain Res 2002; 937(1-2): 58-65.
39- Baulieu,EE., Schumacher M., Koenig H., Jung-Testas I., & Akwa Y. Progesterone as a neurosteroids: action within the nervous system. Cell. Mol. Neurobiol. 1996; 16: 143-154.

40- Gore-Langton.,& Armestrong DT. Follicular steroidogenesis and its control. In Knobill, E., & Neill JD., (eds), The physiology of reproduction.New York, Raven Press, 1994; 571-627.
41- Hall PF. 1994. Testicular steroid synthesis: organization and regulation. In Knobill, E., & Neill JD., (eds), The physiology of reproduction.New York, Raven Press, 1335-1362.
42- Martini L., Celotti F., & Melcangi RC. Testosterone and progesterone metabolism in the central nervous system: cellular localization and mechanisms of control of the enzyme involved. Cell. Mol. Neurobiol 1996; 16: 271-282.
43- Arnold SF., Klotz DM., Collins BM., Vonier PM., Guillet LJ., & McLachlan JA. Synergetic activation of estrogen receptor with combinations of environmental chemicals. Science1996; 272: 1489-1492.
44- Shughrue PJ., Komm B., & Merchenthaler I. The distribution of estrogen receptor-ß mRNA in the rat hypothalamus. Steroids 1996; 61:678-681.
45- Huang Y., Zhu YC., & Yao T. Actions of neuroactive steroids in neurons. Sheng Li Ke Xue Jin Zhan 2002; 33(1): 6-12.
46- Beato M. Gene regulation by steroid hormones. Cell 1989; 5: 335-344. Alonso R., & Lopez-Coviella I. Gonadal steroids and neural function. Neurochemical Research 1998; 23(5); 675-688.
47- McEwen BS. Corticosteroids and hippocampal plasticity. Ann. N.Y. Acad. Sci 1994; 746: 134-144.
48- McEwen BS. How do sex and stress hormones affect nerve cells? . Ann. N.Y. Acad. Sci 1994; 743: 1-18.
49- Wehling M. Nongenomic actions of steroid hormones. TEM 1994; 5: 347-353. Ramirez VD., Zeng J., & Siddique KM. Membrane receptors for estrogen, progesterone and testosterone in the rat brain: fantasy to reality. Cell. Mol. Neurobiol 1996; 16: 175-198.
50- Schumacher M. Rapid membrane effects of steroid hormones : an emerging concept of neuroendocrinology. TINS 1990; 13: 356-361.
51- Joels M., & Klote ER. Control of neural excitability by corticosteroid hormones. TNS 1992; 15: 25-30.
52- Baulieu EE., & Robel P. Non-genomic mechanisms of action of steroid hormones. In Non- reproductive actions of sex steroids (Ciba symposium 191). Chichester, John Wiley & Sons 1995; 24-42.